MOLECULAR PHARMACOLOGY, 23:614–618

Ionization Processes of Some Harmala Alkaloids

KENNETH T. DOUGLAS, RAJIV K. SHARMA, JOANNE F. WALMSLEY, AND ROBERT C. HIDER
Department of Chemistry, University of Essex, Colchester, Essex, CO4 3SQ, United Kingdom

Received May 14, 1982; Accepted December 21, 1982

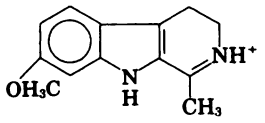
SUMMARY

The ionization and UV-visible spectral properties of some harmala alkaloids have been investigated spectrophotometrically. Harmaline and harmine were found to have pK_a values of 9.55 ± 0.04 and 7.45 ± 0.03, respectively. The ionization of harmalol was characterized by two processes which could be spectrophotometrically isolated from one another, allowing pK_a values of 8.62 ± 0.15 and 11.30 ± 0.23 to be determined. The lower of these was ascribed to the phenolic group and the higher to the enamino site. Support for this assignment lay in the yellow color (λ_{max} 433 nm) at intermediate pH values, which was typical of a formally neutral quinone-methide structure. For harmol, pK_a values of 7.90 and 9.47, reported at 21° by Perrin [*N. Z. J. Sci. Technol.* **38B**:688–694 (1957)], were reassigned with the lower value reflecting phenolic ionization, as opposed to the original literature assignment. Partition coefficients at pH 7.4 (*n*-heptane/water) were determined. The comparative pharmacology of these alkaloids is discussed and related to both their pK_a values and the relative stability of the neutral quinone-methide structure.

INTRODUCTION

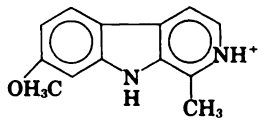
Harmaline alkaloids are finding increasing application in pharmacological studies (1–6) by virtue of their ability to compete for Na^+ sites (7–11), although some members of the group also compete for acetylcholine sites (4) and aromatic amine sites (12, 13). Not surprisingly, they possess different relative affinities for these sites; for instance, harmalol (3) is a more potent inhibitor than harmine (2) toward the high-affinity choline uptake system of synaptosomes (14), whereas the reverse is true for competition at acetylcholine binding sites (4), the ability to induce tremor activity (1), and the tendency to decrease arterial blood pressure (2). The low tremor-inducing ability of harmalol (3) as compared with harmine (2) and harmaline (1) has been associated with its relatively low heptane/water partition coefficient. However, such an explanation is difficult to correlate with the high potency of harmalol (3) toward the Na^+,K^+-activated Mg^{2+}-dependent ATPase, for which the binding site is believed to be on the inside of the membrane (7, 8). Thus it would appear that the range of efficacies with which harmala alkaloids interact with receptors, transport proteins, and enzymes is probably related to subtle chemical differences that exist within the group and not to their differential partition properties. One property that separates the four harmala alkaloids (1–4) is that of pK_a values. For instance, although *1* and *2* are apparently very similar, their pK_a values are reported to be 10.0 and 7.65, respectively (4). Thus whereas harmaline is totally protonated at physiological pH, both charged and uncharged harmine species are present at comparable concentrations. With harmalol (*3*) and harmol (*4*), the situation is more complicated as there are two sites of proton dissociation. Which of these two sites preferentially dissociates could have considerable bearing on the over-all properties of the molecule. For instance, the quinone methide (*5b*) could be a contributing form of harmalol (*3*) at pH 7.0. Should this be the case, then a ready explanation for the differential pharmacological properties of harmine and harmalol would become apparent.

This paper reports the determination and assignment of the pK_a values of four harmala alkaloids (*1–4*), together with a discussion, correlating the spectrum of pharmacological properties associated with these molecules and their predominant structural forms under physiological conditions.



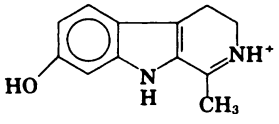
1

Harmaline



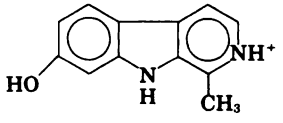
2

Harmine



3

Harmalol



4

Harmol

EXPERIMENTAL PROCEDURES

Materials. The hydrochloride salts of harmalol, harmaline, harmol, and harmine were obtained from Sigma Chemical Company (St. Louis, Mo.). Water was glass-distilled and deionized. Buffers were prepared

0026-895X/83/030614-05$02.00/0

using Analar reagents whenever possible. *n*-Butylamine was redistilled before use.

Methods. Spectrophotometric measurements were made on a Carlo Erba Spectrophotometer, a Pye-Unicam SP8100, or a Tekman Series 634 UV-visible spectrophotometer with a thermostated cuvette compartment using quartz cells of 10-mm pathlength. A Philips PW-9409 pH meter, with the temperature of the sample cell regulated by means of circulating water, was used. All absorbance and pH measurements were made at 25.0° and ionic strength 0.1.

Partition coefficients, determined using *n*-heptane as organic phase, were estimated from absorption measurements after 15 min of incubation with equal volumes of *n*-heptane and aqueous solutions of the drugs (10^{-5} M) buffered to pH 7.4 with Tris-HCl (10 mM).

RESULTS

Harmaline (1). The ionization of harmaline leads to loss of the enamino proton, which causes significant spectral changes: the neutral species absorbs maximally at longer wavelength than the protonated and there is an isosbestic point at 346 nm (see Fig. 1a). The pH dependence of the absorbance at 370 nm reflected the single expected ionization and obeyed the Henderson-Hasselbach equation (see Fig. 2b) for pK_a = 9.49 by least-squares fitting of the pH profile (log-log form). Direct substitution into the appropriate form of the Henderson-Hasselbach equation for data (five points) in the region of the pK_a (pH 8.96–10.39) gives pK_a = 9.55 ± 0.04.

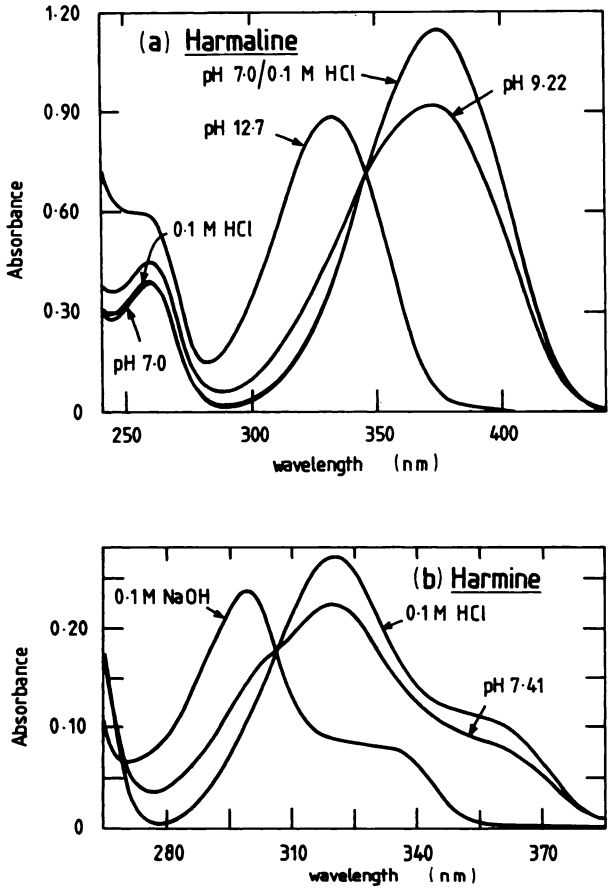


FIG. 1. *Spectra of harmaline and harmine*

a. Spectra of harmaline (3.11×10^{-5} M) at various pH values (25°, ionic strength 0.1) as indicated on the curves. The protonated form absorbs with λ_{max} values of 331 nm ($\epsilon = 2.85 \times 10^4$ M^{-1} cm^{-1}) and 258 nm ($\epsilon = 1.45 \times 10^4$ M^{-1} cm^{-1}). The neutral form (deprotonated) absorbs maximally at λ_{max} 373 nm ($\epsilon = 3.68 \times 10^4$ M^{-1} cm^{-1}), and there is an isosbestic point for the two forms at 346 nm.

b. Spectra of harmine (1.34×10^{-5} M) at various pH values (as indicated) and at 25°, ionic strength 0.1. The protonated form absorbs maximally at 320 nm ($\epsilon = 2.01 \times 10^4$ M^{-1} cm^{-1}) and the deprotonated form at 300 nm ($\epsilon = 1.77 \times 10^4$ M^{-1} cm^{-1}) with a shoulder at ~360 nm, some 40% as intense. The isosbestic point is at 306 nm.

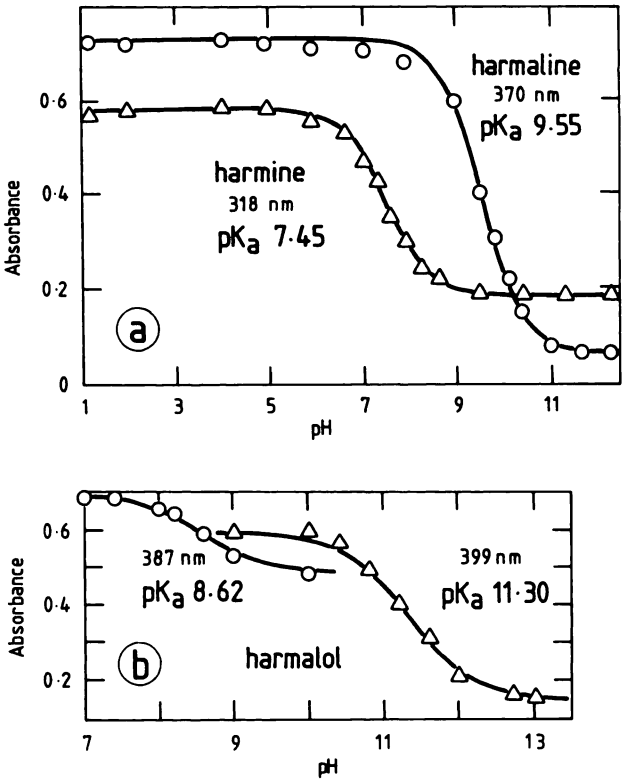


FIG. 2. *Ionization and titration curves*

a. Ionization curves for harmaline and harmine. Points represent experimental absorbances measured at 318 nm for harmine (1.34×10^{-5} M) and 370 nm for harmaline (3.11×10^{-5} M). Lines are theoretical for pK_a values of 7.45 and 9.55 for harmine and harmaline, repsectively, with limiting absorbance values of 0.579 and 0.184 for harmine and 0.724 and 0.065 for harmaline.

b. Titration curves for harmalol (2.77×10^{-5} M). The curve measured at 387 nm is for the K_1 equilibrium ($AH_2 \rightleftharpoons AH^-$) and that at 399 nm is for the K_2 process ($AH^- \rightleftharpoons A^{2-}$). Points are experimental. The line through the data at 387 nm is theoretical for an acid of pK_a = 8.62 with limiting values of A_{387} in acidic and basic media of 0.690 and 0.480, respectively. The line through the 399-nm data is theoretical for an acid of pK_a = 11.30 with limiting values of A_{399} in acidic and basic media of 0.591 and 0.150, respectively.

Harmine (2). Ionization of harmine involved the pyridinium site and was also accompanied by spectral changes. On deprotonation the peak at 320 nm (ϵ 2.01 × 10^4 M^{-1} cm^{-1}) with a shoulder at ~360 nm was reduced in intensity, and a new peak at 300 nm (ϵ 1.77 × 10^4 M^{-1} cm^{-1}) for the neutral molecule appeared at higher pH values (Fig. 1b). The absorbance in the region of 250 nm also decreased markedly on deprotonation, and the isosbestic point for the acidic and basic forms was 306 nm. Plotting A_{318} (the absorbance at 318 nm) versus pH gave a good fit of data to the Henderson-Hasselbach equation (Fig. 2a) with pK_a = 7.50 (by least-squares fitting of the logarithmic form of the pH profile) and pK_a = 7.45 ± 0.03 by direct substitution (six pH values, pH 6.63–8.22) into the Henderson-Hasselbach equation.

Harmalol (3). The pH dependence of the spectra of harmalol was complex and revealed two ionization processes; i.e., the ionizations fitted the scheme $AH_2 \overset{K_1}{\rightleftharpoons} AH^- \overset{K_2}{\rightleftharpoons} A^{2-}$. The first ionization occurred in the region pH 7–9 (Fig. 3a). Loss of the first proton from the AH_2 form (*3*) of harmalol led to the appearance of a new band at 433 nm. As the solution was made more alkaline (pH 10–12), a second proton was lost and the 433-nm band disappeared (Fig. 3b). Thus, the ionization of harmalol (AH_2) occurs in two stages. The AH_2 species absorbs maximally at 370 nm (ϵ 32,200 M^{-1} cm^{-1}), the AH^- species at 433 nm ($\epsilon \sim$ 32,500 M^{-1} cm^{-1}) and the A^{2-} species at 362 nm (ϵ 27,700 M^{-1} cm^{-1}). The presence of the AH^- form of harmalol is characterized by an intense yellow color (corresponding to the 433-nm peak). When the absorbance of harmalol at 430 nm was plotted as a function of pH, a skewed bell-shaped profile resulted with a maximum at ~ pH 9.8, the pH at which the concentration of the AH^- form is maximal. Although pK_a values for the $AH_2 \rightleftharpoons AH^-$ and $AH^- \rightleftharpoons A^{2-}$ equilibria can be extracted from such data, it is easier to make use of the observed isosbestic points for these K_1 and K_2 processes. The isosbestic point for the K_1 process (Fig. 3a) was at ~399 nm and that for the K_2 process was at ~387 nm (Fig. 3b). Thus, we could study the K_1 process by measuring absorbance changes in the appropriate pH region at 387 nm, the isosbestic point for the K_2 equilibrium. Conversely, the K_2 process could be isolated spectrophotometrically by working at 399 nm. The results of this approach are shown in Fig. 2b, in which are plotted absorbances at 387 nm and 399 nm for harmalol at various pH values. The pK_a values thus determined for harmalol were $pK_1 = 8.62 \pm 0.15$ and $pK_2 = 11.30 \pm 0.23$.

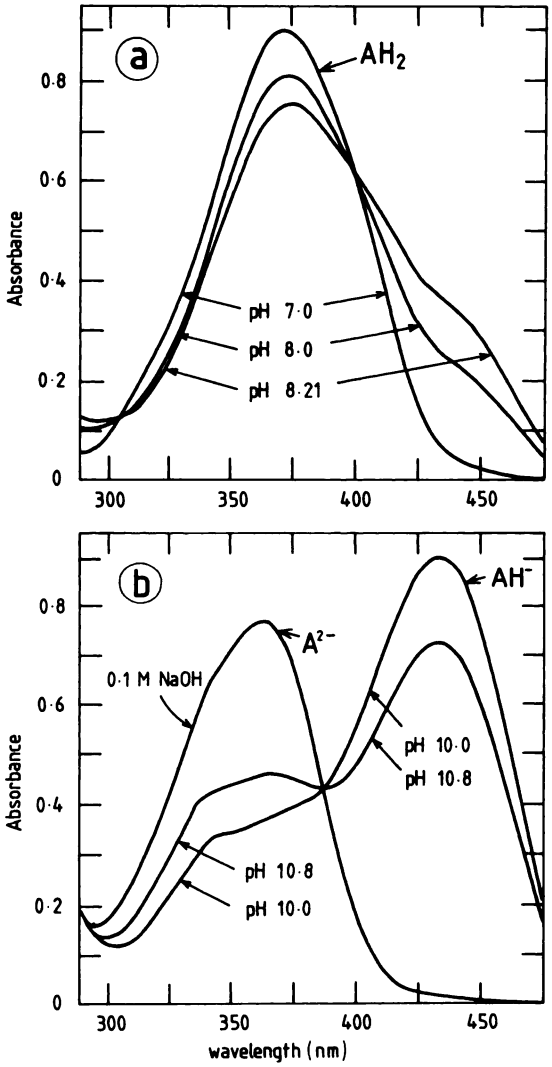


FIG. 3. *pH-Dependent spectra of harmalol*

a. pH-Dependent spectra (at 25°, 0.1 ionic strength) of harmalol (2.77×10^{-5} M) showing the diprotonated form AH_2, which absorbs maximally at 370 nm (ϵ = 32,200 M^{-1} cm^{-1}) and isosbestic points for the $AH_2 \overset{K_1}{\rightleftharpoons} AH^-$ equilibrium at 305 nm and 399 nm.

b. pH-Dependent spectra (at 25°, 0.1 ionic strength) of harmalol (2.77×10^{-5} M) showing the A^{2-} form (λ_{max} = 362 nm, ϵ = 27,700 M^{-1} cm^{-1}) and AH^- form (λ_{max} = 433 nm, ϵ = 32,500 M^{-1} cm^{-1}) with isosbestic points for the $AH^- \overset{K_2}{\rightleftharpoons} A$ = equilibrium at 289 nm and 387 nm.

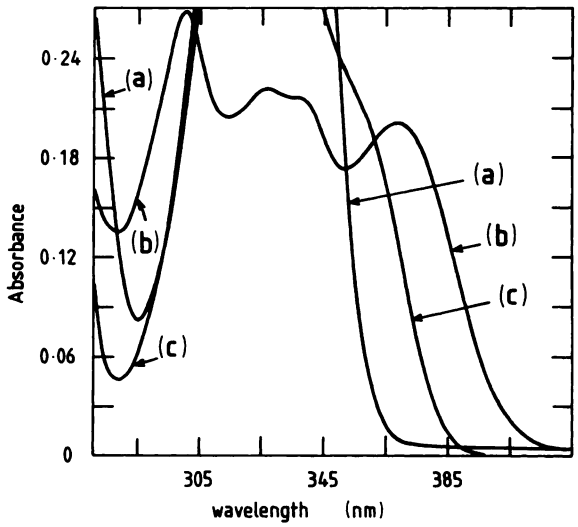


FIG. 4. *UV spectra of harmol*

UV spectra of harmol (28.4 μM) in (a) 0.1 M sodium hydroxide solution; (b) pH 8.70, 0.01 M Tris buffer; and (c) pH 5.00, 0.1 M sodium acetate buffer at 25°.

TABLE 1

Partition coefficients (K_{part}) for harmala alkaloids between n-heptane and aqueous phases

Alkaloid	K_{part} (*n*-heptane)	
	Zetler *et al.* (1)	This work
Harmaline (*1*)	0.05	0.11
Harmine (*2*)	0.31	0.50
Harmalol (*3*)	0.01	0.03
Harmol (*4*)	—	0.36

Harmol (4). The spectra of harmol at pH 5.00, pH 8.70, and in 0.1 M sodium hydroxide are shown in Fig. 4. No peaks were detected at wavelengths greater than 425 nm. At pH 8.7 a peak appeared at 369 nm (ϵ 7041): this peak was not evident at higher or lower pH values. Perrin (15) described a similar peak at pH 8.5 for harmol but did not discuss its assignment; however, he did report that the first and second acid dissociation constants were 7.90 and 9.47 at 21°, indicating that this peak belongs to the "free base" form (i.e., *4* with one proton removed).

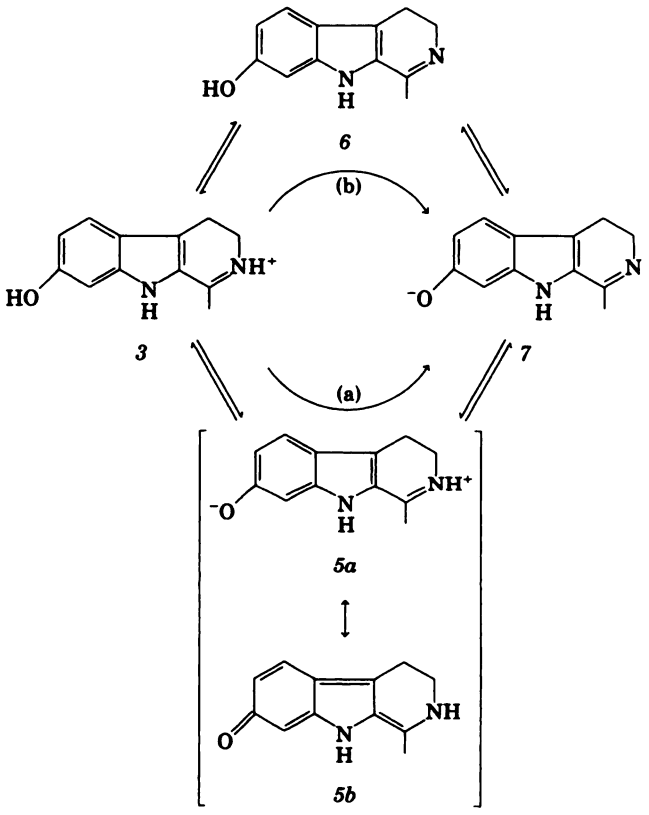
Partition coefficients. The partition coefficients (K_{part}) for the alkaloids *1–4* are presented in Table 1 together

with those reported by Zetler *et al.* (1). The values are closely related to the net charge of the alkaloid at pH 7.4; harmaline and harmalol possess K_{part} values lower than those of harmine and harmol.

DISCUSSION

Assignment of dissociation constants. Harmalol can, in principle, ionize as depicted in Scheme 1. In Pathway a the phenolic proton is first lost, followed by the enamino proton; in Pathway b, the protons are lost in the opposite order. An analogous scheme can also be written for harmol.

Assignment of the observed ionizations to molecular events under such circumstances can be difficult. Fortunately, significant spectral changes occur in this system on successive ionizations. The formation of AH^- (*6*, *5a*, or *5b*) for harmalol (*3*) is accompanied by a new spectral band in the visible range at 433 nm (ϵ 32,500 M^{-1} cm^{-1}). This band is lost again as the monoanion (*7*) is produced at yet higher pH. This shift to the visible range is typical of increased delocalization in a molecule upon ionization. If the appearance of the 433-nm band were caused by the process *3* → *6*, a suitable spectral comparison would be with harmaline (*1*). However, for harmaline, deprotonation, to give the analogue of *6*, leads to a blue shift from ~370 to ~330 nm. A similar blue shift occurs for harmine on deprotonation. Thus deprotonation of harmalol to *5a* and *5b* appears likely. In further support of the assignment of the pK_1 (~8.62) to phenolic ionization lies in the chemical nature of *5b*, one of the canonical forms of the AH^- species for harmalol. It is formally analogous to a quinone methide, stable members of which are usually yellow or highly colored (16, 17).



SCHEME 1

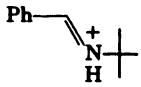
TABLE 2

Acid dissociation constants for harmala alkaloids at 25°, 0.1 ionic strength, except where noted

Alkaloid		Functional group	pK_a values	Reference
Harmaline	(*1*)	NH	9.55	This work
			10	4
			9.8	18
				19
Harmine	(*2*)	NH	7.50	This work
			7.65	4
			8.30	19
Harmalol	(*3*)	NH	11.30	This work
			11.0	4
		OH	8.62	This work
Harmol	(*4*)	NH	9.51	This work
			7.90	15
		OH	7.86	This work
			9.47	15

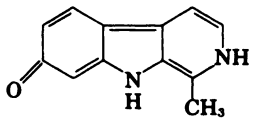
On this basis we assign[1] the pK_a of 8.62 to the phenolic ionization and that of 11.30 to the nitrogen site (Table 2).

The pK_a of 8.62 is low for a phenol, being approximately that of *m*-nitrophenol (20) (pK_a = 8.39). Indeed, the pK_a of this function gives an idea of the degree of stabilization confirmed by the "quinone-methide" (*5b*) contribution achieved by such ionization. The stability of this formally neutral species of harmalol (*5a*, *5b*) is underlined by the value of 11.30 for pK_2, which is extremely high for a Schiff base. An apparently analogous benzylidene derivative (*8*) (21) has pK_a = 6.7. Relative to harmaline (*1*), the pK_a for the nitrogen site in harmalol is raised some 1.5 pK units.



8

By analogy with harmalol, the AH^- absorption (λ_{max} 369 nm) being shifted to wavelengths longer than those for AH_2 or A^{2-}, a quinone-methide type contribution (*9*) also can be proposed for harmol. In agreement with our results (Fig. 4), such a structure would be expected to contribute less to the



9

[1] In furtherance of a referee's suggestion we have measured spectrophotometrically the pK_a values for harmalol in mixed organic/aqueous [viz., 20% acetonitrile-water (v/v)] media. The pK_{app} values obtained were 8.75 ± 0.10 (for eight pH_{app} values by insertion into the Henderson-Hasselbach equation) and 11.1 ± 0.04 (five determinations by Henderson-Hasselbach substitution) studied at the appropriate isosbestic points of 391 and 406 nm, respectively, determined in these media. These do not differ significantly from the values determined in purely aqueous media. The use of water/low dielectric solvent mixtures has been used in enzymology to decide whether enzyme ionizations represent cationic or neutral acid deprotonations. However, in the present case the lack of change in apparent pK_a values with solvent composition cannot distinguish between Routes b and a (*3* → *5* → *7*) if species *5* (as seems likely from the yellow color) is better represented by the formally neutral hybrid *5b*.

properties of the AH^- form than its analogue for harmalol, as it involves partial destruction of the aromatic character of the six-membered *N*-ring. Again as for harmalol, the lower pK_a for harmalol is ascribed to phenolic dissociation and the higher pK_a to pyridinium dissociation. These assignments are different from those previously made by Perrin (15).

Comparative pharmacology of the harmala alkaloids, 1–4. With some pharmacological processes there is little difference in the inhibitory influence of the four alkaloids *1–4* [for instance, toward the benzodiazepine receptor (22) serotonin uptake by synaptosomes (23), and the sodium pump (7)]. Presumably, the primary interaction in these examples occurs via the cationic nitrogen atom. However, with some receptors there is a clear difference in the inhibitory potency. With monoamine oxidase, for instance, both harmaline and harmine are far more powerful inhibitors than are both harmalol and harmol [EC_{50} values of 6×10^{-8} and 8×10^{-8} M as compared with 1×10^{-5} and 6×10^{-6} M, respectively) (24)], and tremorigenic potency is effectively abolished by the conversion of the methoxy to a hydroxy function (1, 25, 26). Similar findings have been reported concerning the indirect block of ouabain-induced smooth muscle contractions (5), a phenomenon possibly related to markedly different cardiovascular actions of harmaline and harmalol (2). These differential activities are not readily explained in terms of K_{part} and pK_a values. Singbartl *et al.* (26) have come to a similar conclusion concerning K_{part} values. Harmine and harmol have the higher K_{part} values, which are most likely related to their relatively low pK_a values. Clearly another reason for the large differences in behavior of the phenolic and methoxy harmaline derivatives must exist, a likely possibility being the manner with which the compounds interact with protein binding sites. The one outstanding difference between the two classes is the ability of the phenolic alkaloids (*3* and *4*) to form the formally noncharged quinone-methide species (*5b* and *8*). The formation of these mesomers could be favored by the environment of the protein binding site and as consequence lead to a lower affinity due to the neutralization of the normally positively charged nitrogen atoms.

An interesting collorary to this conclusion is that harmol, like harmalol (14), might be anticipated to be a more powerful inhibitor of choline uptake than both harmaline and harmine.

REFERENCES

1. Zetler, G., G. Singbartl, and L. Schlosser. Cerebral pharmacokinetics of tremor-producing harmala and Iboga alkaloids. *Pharmacology (Basel)* **7**:237–248 (1972).
2. Aarons, D. H., G. V. Rossi, and R. F. Orzechowski. Cardiovascular actions of three harmala alkaloids: harmine, harmaline, and harmalol. *J. Pharm. Sci.* **66**:1244–1248 (1977).
3. Carpentier, R. G. Effects of harmine on transmembrane potentials of guinea-pig atrial muscle. *Br. J. Pharmacol.* **69**:561–564 (1980).
4. Hider, R. C., L. Smart, and M. S. Suleiman. The effect of harmaline and related β-carbolines on the acetylcholine-stimulated contractions of guinea-pig ileum. *Eur. J. Pharmacol.* **70**:429–436 (1981).
5. Hider, R. C., L. Smart, and M. S. Suleiman. The effect of harmaline and related harmala alkaloids on ouabain-stimulated contractions of the guinea-pig ileum. *Eur. J. Pharmacol.* **71**:87–92 (1981).
6. Costall, B., D. M. Kelly, and R. J. Naylor. The importance of 5-hydroxytryptamine for the induction of harmine tremor and its antagonism by dopaminergic agonists assessed by lesions of the midbrain raphe nuclei. *Eur. J. Pharmacol.* **35**:109–119 (1976).
7. Canessa, M., E. Jaimovich, and M. de la Fuente. Harmaline: a competitive inhibitor of Na ion in the $(Na^+ + K^+)$-ATPase system. *J. Membr. Biol.* **13**:263–282 (1973).
8. Dunn, M. J., and W. Hunt. The effects of harmaline on sodium transport in human erythrocytes: evidence in favour of action at interior sodium-sensitive sites. *J. Pharmacol. Exp. Ther.* **193**:903–909 (1975).
9. Buclon, M., F. V. Sepulveda, and J. W. L. Robinson. The reversibility of the inhibition of intestinal amino-acid transport by harmaline. *Naunyn-Schmiedebergs Arch. Pharmacol.* **298**:57–59 (1977).
10. Mahmood, A., and F. Alvarado. Harmaline interaction with sodium-binding sites in intestinal brush border sucrase. *Biochim. Biophys. Acta* **483**:367–374 (1977).
11. Lea, T. J., and C. C. Ashley. Harmaline distribution in single muscle fibres and the inhibitions of sodium efflux. *Biochim. Biophys. Acta* **664**:74–81 (1981).
12. Tsai, T. H., and W. W. Fleming. Antagonism of monoamine oxidase inhibitors against norepinephrine, acetylcholine and potassium in the isolated nictitating membrane of the cat. *J. Pharmacol. Exp. Therapeut.* **148**:40–47 (1965).
13. Robertson, H. A. Harmaline-induced tremor: the benzodiazepine receptor as a site of action. *Eur. J. Pharmacol.* **67**:129–132 (1980).
14. Smart, L. S. Competitive inhibition of sodium-dependent high affinity choline uptake by Harmala alkaloids. *Eur. J. Pharmacol.* **75**:265–270 (1981).
15. Perrin, D. D. The dissociation constants of perlolidene and harmol. *N. Z. J. Sci. Technol.* **38B**:688–694 (1957).
16. Wagner, H.-U., and R. Gompper. Quinone methides, in *The Chemistry of the Quinonoid Compounds* (S. Patai, ed.). John Wiley and Sons, New York, 1145–1178 (1974).
17. Nakayama, J., K. Yamashita, M. Hoshino, and T. Takemasa. The synthesis of highly stable *o*- and *p*-quinone methides. *Chem. Lett.* 789–797 (1977).
18. Orlow, I. E. Bestimmung der Dissoziationskonstanten von Harmalin und Harmin. *Bull. Wiss. Chem. Pharm. Forsch. Inst.* 277–281 (1931).
19. Duportail, G., and H. Lami. Studies of the interaction of the fluorophores harmine and harmaline with calf thymus DNA. *Biochim. Biophys. Acta* **402**:20–30 (1975).
20. Jencks, W. P., and J. Regenstein. Dissociation constants of acids and bases, in *Handbook of Biochemistry*, Ed. 2 (H. A. Sober, ed.). Chemical Rubber Publishing Company, Cleveland, Ohio, Section J-187 (1970).
21. Cordes, E. H., and W. P. Jencks. On the mechanism of Schiff base formation and hydrolysis. *J. Amer. Chem. Soc.* **84**:832–837 (1962).
22. Rommelspacher, H., C. Nanz, H. O. Barbe, K. J. Fehske, W. E. Müller, and U. Wollert. 1-Methyl-β-carboline (harmane), a potent endogenous inhibitor of benzodiazepine receptor binding. *Naunyn-Schmiedebergs Arch. Pharmacol.* **314**:97–100 (1980).
23. Buckholtz, N. S., and W. O. Boggan. Inhibition by β-carbolines of monoamine uptake into a synaptosomal preparation: structure-activity relationships. *Life Sci.* **20**:2093–2100 (1977).
24. Buckholtz, N. S., and W. O. Boggan. Monoamine oxidase inhibition in brain and liver produced by β-carbolines: structure-activity relationships and substrate specificity. *Biochem. Pharmacol.* **26**:1991–1996 (1977).
25. Gunn, J. A. Relations between chemical constitution, pharmacological actions and therapeutic uses in the harmine group of alkaloids. *Arch. Int. Pharmacodyn. Ther.* **50**:379–396 (1935).
26. Singbartl, G., G. Zetler, and L. Schlosser. Structure-activity relationships of intracerebrally injected tremarigenic indole alkaloids. *Neuropharmacology* **12**:239–244 (1973).

Address correspondence to: Dr. K. T. Douglas, Department of Chemistry, University of Essex, Colchester, Essex CO4 3SQ, United Kingdom.